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Qualitative screening for new psychoactive substances in wastewater collected during a city festival using liquid chromatography coupled to high-resolution mass spectrometry

Ana Causanilles [a, b, 1], Juliet Kinyua [c, 1], Christoph Ruttkies [d], Alexander L.N. van Nuijs [c], Erik Emke [a], Adrian Covaci [c], Pim de Voogt [a, b, *]

[a] KWR Watercycle Research Institute, Chemical Water Quality and Health, P.O. Box 1072, 3430 BB, Nieuwegein, The Netherlands
[b] Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94248, 1090 GE, Amsterdam, The Netherlands
[c] Toxicological Centre, Department of Pharmaceutical Sciences, Campus Drie Eiken, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium
[d] Leibniz Institute of Plant Biochemistry, IPB Halle, Department of Stress and Developmental Biology, Weinberg, Halle, Germany

HIGHLIGHTS

- Wastewater collected during city-wide festival to track recreational substances use.
- Combination of independent and dependent data acquisition with LC-HRMS.
- Qualitative screening and reporting hits of NPS is more useful.
- Results suggest prevalence of classical drugs and low NPS use.

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ABSTRACT

The inclusion of new psychoactive substances (NPS) in the wastewater-based epidemiology approach presents challenges, such as the reduced number of users that translates into low concentrations of residues and the limited pharmacokinetics information available, which renders the choice of target biomarker difficult. The sampling during special social settings, the analysis with improved analytical techniques, and data processing with specific workflow to narrow the search, are required approaches for a successful monitoring. This work presents the application of a qualitative screening technique to wastewater samples collected during a city festival, where likely users of recreational substances gather and consequently higher residual concentrations of used NPS are expected. The analysis was performed using liquid chromatography coupled to high-resolution mass spectrometry. Data were processed using an algorithm that involves the extraction of accurate masses (calculated based on molecular formula) of expected *m/z* from an in-house database containing about 2,000 entries, including NPS and transformation products. We positively identified eight NPS belonging to the classes of synthetic cathinones, phenethylamines and opioids. In addition, the presence of benzodiazepine analogues, classical drugs and other licit substances with potential for abuse was confirmed. The screening workflow based on a database search was useful in the identification of NPS biomarkers in wastewater. The findings highlight the specific classical drugs and low NPS use in the Netherlands. Additionally, *meta*-chlorophenylpiperazine (mCPP), 2,5-dimethoxy-4-bromophenethylamine (2C-B), and 4-fluoroamphetamine (FA) were identified in wastewater for the first time.

1. Introduction

A current trend in analytical and environmental chemistry is the chemical analysis of raw wastewater in order to identify specific biomarkers that could inform on the health and lifestyle of the population living in the catchment area under study (Daughton,

* Corresponding author. Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94248, 1090 GE, Amsterdam, The Netherlands
E-mail address: w.p.devoogt@uva.nl (P. de Voogt).
[1] Joint first authorship.

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2001). This approach, named wastewater-based epidemiology (WBE), has been successfully applied in revealing the use of illicit substances (Ort et al., 2014; Thomas et al., 2012) and other licit substances, such as pharmaceuticals (Baz-Lomba et al., 2016; Causanilles et al., 2016), alcohol (Ryu et al., 2016), tobacco (Castiglioni et al., 2014), stress biomarkers (Ryu et al., 2015), and more recently new psychoactive substances (NPS) (Bade et al., 2017; Borova et al., 2015; González-Mariño et al., 2016; Kinyua et al., 2015a; Senta et al., 2015).

NPS are psychotropic drugs that produce similar effects to those produced by illicit substances, and are not directly controlled by international conventions (Reid and Thomas, 2016b). They may pose a public health threat, because there is no scientific evidence of their pharmacokinetics, recommended dose, effects or safety. Furthermore, they are easily acquired through the internet and smart shops where they are sold under various product labels with often misleading information. To date, more than 560 NPS have been reported (EMCDDA, 2016). Their monitoring and control is a challenging task because the NPS market is very dynamic with analogues constantly emerging to satisfy consumers' demands and avoid criminalization.

Typically, WBE studies focus on target analysis of well-known biomarkers that are present at a sufficient concentration to be detected and quantified. In the case of NPS, several challenges may arise: the choice of target biomarker is difficult; analytical standards are high-priced and in some cases not available, particularly for metabolites. These recreational substances are mostly consumed to a lesser extent compared to popular substances (cocaine, ecstasy), which will be translated into very low ng L^{-1} residue concentrations in wastewater. As such, recent WBE studies that focused on target screening of NPS with available reference standards using low and high resolution MS have shown that extremely low levels are detected or none at all (Bade et al., 2017; Borova et al., 2015; González-Mariño et al., 2016; Kinyua et al., 2015a; Senta et al., 2015). In contrast, studies that applied qualitative screening techniques based on high-resolution mass-spectrometry (HRMS) to biological matrices from individuals, namely blood and urine, (Andreasen et al., 2015; Kinyua et al., 2015b; Negreira et al., 2016b; Sundström et al., 2015), or collective pooled urine from festivals (Archer et al., 2013, 2014; Kinyua et al., 2016) have detected and identified several NPS and their metabolites. The results from the previously cited works stress the importance of different sample matrices, sampling locations and the role of improved analytical techniques to track NPS use. Pooled urine from recreational areas has the advantage that levels of drug biomarkers are higher and easier to detect compared to wastewater (Kinyua et al., 2016; Mardal et al., 2017). However, the collection of pooled urine may likely miss samples from female users (Kinyua et al., 2016). Wastewater influent samples collected at a wastewater treatment plant (WWTP) are representative for populations connected to the sewer system and would provide useful information about an entire community's use of NPS within a catchment area if advanced analytical techniques are applied. However, consumption by the population, and hence concentrations of NPS in wastewater, are much lower than those of the more traditional illicit drugs and the daily wastewater levels may thus not be sufficiently high to allow detection. A modification aimed at gathering qualitative information on NPS use by targeting sampling at some social settings (festivals or events) where substance use is elevated would increase the likelihood of successfully identifying and detecting NPS (Reid and Thomas, 2016a). Another powerful improvement which can be applied when no reference standards are available to confirm mass spectra and retention time information is the use of HRMS using the "suspect screening" approach, which involves extraction of the exact masses of expected ions $[M+H]^+$ or $[M-H]^-$ from the acquired data (Hernández et al., 2016; Kinyua et al., 2015b; Krauss et al., 2010). Fundamentally, we can cast a wider net for screening of NPS by creating a suspect list that includes all potential biomarkers of interest.

In the Netherlands, NPS appear to be used at a lower rate compared to other countries, more in particular the UK, because of the high quality, low price and availability of classical drugs such as cocaine and 3,4-methylenedioxymethamphetamine (MDMA) (Hondebrink et al., 2015). It is noteworthy to mention that amphetamine-type stimulants are easy to acquire because The Netherlands and Belgium are the most important European production areas (EMCDDA and Europol, 2016). Thus far, NPS have been found as adulterants or as a replacement of classical drugs without users' awareness. However, their use is increasing progressively in recent years as drug of choice, as data from the Poisons Information Centre show (Hondebrink et al., 2015).

The aims of the present study were to: i) analyse wastewater collected during a special social setting with a suspect screening approach; ii) elucidate the identity of NPS and provide a qualitative snapshot of recreational substances used during a festival in the Netherlands; iii) discuss the challenges in applying a suspect screening approach to wastewater samples. For this purpose, we used liquid chromatography coupled to HRMS for the screening of eight 24-h composite raw wastewater samples collected at the WWTP serving the catchment area of Amsterdam in 2012 and 2014, during festivals that brought approximately 300,000 visitors to the city.

2. Materials and methods

2.1. Sample collection

Eight 24-h flow-dependent influent composite samples were collected after the sand trap at the main WWTP serving the city of Amsterdam, representing 769,000 inhabitants (according to census data). The sampling campaign was performed in the summer of 2012 and 2014 just prior to and during a festival that attracted ~300,000 visitors to the city. Such festivals are key sites for drug use among young people (Dilkes-Frayne, 2016). Four samples corresponding to 24-h composite samples from Thursday to Sunday were collected in both years. WWTP characteristics and sample details can be found in Table SI-1 (in Supplementary Information).

2.2. Sample treatment

Samples were stored in HDPE containers and frozen immediately after collection at −20 °C until analysis. Samples were thawed at 4 °C for 24 h, and after homogenization, a 50 mL aliquot was filtered on glass microfiber filters GF/A (1.6 μm). Solid-phase extraction (SPE) was performed with Oasis HLB cartridges (150 mg, 6 cc) pre-conditioned with 8 mL of methanol and 8 mL of ultra-pure water. After sample loading, the SPE cartridge was washed with 4 mL of ultra-pure water and vacuum dried for 30 min. After elution with 8 mL methanol, the eluate was evaporated to dryness with a gentle nitrogen stream in a water bath at 35 °C. The final extract was reconstituted to 250 μL water/methanol 90:10, v/v.

2.3. Instrumental analysis

Extracts were analysed twice by liquid chromatography coupled to high-resolution mass spectrometry: (i) with an Agilent LC-QTOFMS at the Toxicological Centre at the University of Antwerp, Belgium; and (ii) with a Thermo LC-LTQ-Orbitrap at the KWR Watercycle Research Institute in Nieuwegein, The Netherlands.